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2682-Pos Board B374
Calcium and cAMP Dynamics in Pituitary Lactotrophs
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Calcium dependent exocytosis from pituitary lactotrophs is known to be regulated by G protein coupled receptors (GPCRs) that regulate intracellular [cAMP]. We have used fura-2 measurements of intracellular calcium concentration along with both population biochemical cAMP measurements and single cell FRET-based cAMP measurements to investigate the relationship between [Ca^{2+}]i and cAMP in clonal rat MMQ lactotrophs. Both VIP and PACAP activation of GPCR coupled to Gs lead to a Ca^{2+} influx as evident by increase in Ca^{2+}-oscillations. Bypass of GPCRs with the adenylyl cylclase activator forskolin also resulted in strong in increase in Ca^{2+} oscillations consistent with measured forskolin-induced increase in [cAMP]. Pharmacological activation of PKA using 6-Bn-cAMPor activation of EPAC using 8-cpt-cAMP each resulted in increase in Ca^{2+} oscillations indicating a role for both cAMP binding proteins in control of calcium dynamics although with differing lag times likely reflecting distinct signaling pathways. The broad-spectrum cAMP phosphodiesterase (PDE) inhibitor IBMX caused an increase in Ca^{2+} influx without delay. The PDE3 inhibitor milrinone caused a strong increase in Ca^{2+} after a delay whereas the PDE4 inhibitor rolipram caused an immediate increase in Ca^{2+}. FRET-based measurements of [cAMP] are being used to further analyze these differences. These results suggest a possible different relationship between the various PDE sensitive pools of cAMP and the L-type Ca^{2+}-channels responsible for Ca^{2+} influx as well as distinct roles for PKA and EPAC in control of Ca^{2+} dynamics and prolaction secretion.

2683-Pos Board B375
Non-Linear Signal Propagation through Multicellular Chains
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The use of chemical signaling is a primary method for the cells to process environmental cues. Chemosensing by the cells allows them to perceive these chemical stimuli and respond in a manner to maintain normal functionality. It has been shown that various cell types respond to external stimuli by producing calcium ion spikes that act as a signaling agent to nearby cells. In this manner, important system information can be conveyed to a large number of cells without relying on individual sensation.
In this study we use fibroblast cells to explore the biophysical rules underlying the multicellular calcium wave propagation mediated by gap junctions. Using pressure-regulated microfluidics, we are able to control the temporal profile of chemical stimuli, which triggers collective calcium dynamics of fibroblast cell chains patterned by microfluidics. Compared to previous studies, we have tight control over the cell-stimulus interface at sub-cellular resolution as well as the cell morphology through the microfluidics channels. We study the calcium wave characteristics, such as the speed, dispersion, and dissipation as a way to understand the band filtering property of the gap junctions. We also construct cell wires with cancer cell inserts, which disrupt the normal intercellular connectivity. These experiments allow us to construct an effective model that captures the heterogeneity of cell responses, cell-cell connections, and has electronics analogies.

2684-Pos Board B376
The Network Characteristics and Spatial-Temporal Dynamics of Collective Chemosensing
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The collective chemosensing of nonexcitable mammalian cells involves a biochemical network that features gap junction communications and heterogeneous single cell activities. To understand the integrated multicellular chemosensing, we study the calcium dynamics of micropatterned fibroblast cell colonies in response to adenosine triphosphate (ATP) stimulation. We find that the cross-correlation function between the responses of individual cells decays with topological distance as a power law for large colonies and much faster for smaller colonies. Furthermore, the strongly correlated cell pairs tend to form clusters and are more likely to exceed the percolation threshold. At a given topological distance, the cross-correlations exhibit characteristics of Poisson distributions, which allows us to estimate the unitary conductance of a single gap junction which is in good agreement with direct experimental measurements.

2685-Pos Board B377
Epithelial Ca^{2+} Model Prediction: Suramin Enhances P2Y2 Receptor Desensitization by Accelerating their Phosphorylation Rate
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Ca^{2+} signaling is essential to most biological functions. To understand Ca^{2+} signaling in retinal pigment epithelium (RPE), we constructed a computational model of Ca^{2+} wave propagation in ARPE-19 cells based on Ca^{2+} imaging measurements after mechanical stimulation. We exploited the extensive experimental material from Abu Khamidakh et al. 2013 Exp Eye Res. The model relied on the assumption that cells experience different conditions depending on their location with respect to the stimulation site. In this context, the model was used to simulate the measured Ca^{2+} waves and predict the drug effects only by changing the appropriate environmental parameters or drug target components.
Our model reproduced the measured Ca^{2+} dynamics in control cells and in cells after α-glycyrrhetinic acid treatment. As an example of model usage, the model was applied to study suramin inhibition mechanisms in P2Y2 receptors. The model suggested that the suramin inhibition of Ca^{2+} signal propagation may be related to increased P2Y2 receptor phosphorylation rate: the higher phosphorylation rate causes faster desensitization of the receptors after ligand binding shortening the duration of the Ca^{2+} elevation. The modeling results showed that a relatively larger inhibition occurs with higher Ca^{2+} waves near the site of mechanical stimulation.
In summary, the model predicted suramin drug effects on P2Y2 receptors suggesting that suramin enhances their desensitization by accelerating the phosphorylation rate. This intriguing modeling result requires further investigation and needs to be confirmed experimentally for a deeper understanding of the biological mechanisms related to the phenomenon. In general, to date, this is the first mathematical model of Ca^{2+} signaling in ARPE-19 cells and one of the first in general to model epithelial Ca^{2+} dynamics presenting also predictions about the drug modified parameters.

2686-Pos Board B378
Calcium Release from Acidic Stores Modulate Fluid and Protein Secretion in the Salivary Gland
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Autonomic input to salivary glands controls the rate of salivary flow by regulating the activities of ion channels and transporters that induce fluid and protein secretion. This control is achieved by signaling complexes that generate the complex patterns of cytosolic Ca^{2+} observed in acinar cells. Most work to date in these cells has focused on canonical intracellular Ca^{2+} release channels resident on the endoplasmic reticulum. In the current study we identified acidic organelles as an additional releasable Ca^{2+} store in mouse parotid salivary gland. The large, protein-containing secretory granules were found to be the major subcellular acidic organelle. However, the role that acidic Ca^{2+} stores play in these cells in the triggering or globalization of receptor mediated Ca^{2+} signals has not yet been investigated. Treatment with bafilomycin A1, a specific inhibitor of vacuolar-type H^{+}-ATPase, altered the amplitude and kinetics of Ca^{2+} dynamics evoked by low level muscarinic receptor activation but not by stronger stimulations. In contrast bafilomycin treatment nearly abolished protein exocytosis regardless of stimulation intensity. In addition, using acinar cell clusters prepared from mouse salivary gland in combination with flash photolysis and high-speed fluorescence imaging we demonstrated that caged-nicotinic acid adenine dinucleotide phosphate (NAADP) functions as a highly potent Ca^{2+} mobilizing messenger that initiates or sensitizes agonist-stimulated Ca^{2+} signals in these cells. Consistent with a role for NAADP-mediated signaling in acinar cell function, treatment with the NAADP inhibitor BZ194 altered agonist evoked Ca^{2+} signals similar to bafilomycin treatment. This work implicates neurotransmitter-evoked rises in NAADP as a signaling pathway relevant for salivary gland function and identifies a potential target for augmenting function for patients suffering reduced saliva formation.

Calcium Fluxes, Sparks, and Waves II

2687-Pos Board B379
Latency to the Onset of Calcium Waves in Cardiac Myocytes is Predicted by Criticality Theory
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